Quantitative Microconversion of Semicarbazones into 2,4-Dinitrophenylhydrazones

During the course of the development of a method for the direct quantitative isolation of the 2,4-dinitrophenylhydrazone derivatives of carbonyl compounds from fats and oils,[1] it was necessary to have on hand pure parent carbonyls in order to standardize the method. The latter, however, could not always be prepared using conventional purification techniques and thus it was necessary to resort to some other means for obtaining them. It was found that the easily purified semicarbazone derivatives could be readily and quantitatively transformed into the corresponding 2,4-dinitrophenylhydrazone at the μmole level. In view of its simplicity, it was felt that the method might also find application to other problems pertaining to carbonyl microanalysis.

Exchange reaction of semicarbazones with 2,4-dinitrophenylhydrazine has been used in the past by Naves and his associates[2–7] and by others.[8–10] However, no study has been described on the applicability of the exchange reaction to the four commonly occurring aliphatic carbonyl classes, i.e., methyl ketones, saturated aldehydes, 2-enals, and 2,4-dienals. Moreover, no attempt has been made to apply the reaction quantitatively at the micro level.

Experimental

Preparation of Semicarbazones. Semicarbazones were prepared in the usual manner[11] and the derivatives recrystallized to constant melting point. Stock solutions of the derivatives were made in carbonyl-free methylene chloride[12] to contain 1.5–3.0 μmoles per milliliter. An aliquot of this solution was diluted with carbonyl-free hexane[12] so that the methylene chloride concentration was 5% or less.

Conversion of Semicarbazones to 2,4-Dinitrophenylhydrazones. A column of celite impregnated with 2,4-dinitrophenylhydrazine, phosphoric acid, and water prepared according to Schwartz and Parks[12] was flushed with 50 ml. of carbonyl-free benzene using air pressure, and then with carbonyl-free hexane until the effluent emerged colorless. The hexane-methylene chloride solution of the semicarbazone was then permitted to flow over the column at a rate not exceeding 50 ml. per hour. When the last of the solution had entered the column, the sides of the tube were washed with a few milliliters of carbonyl-free hexane. The latter was permitted to drain into the column and an additional 15 ml. of hexane was added and allowed to flow into the column at the natural flow rate. Thereafter, the column was washed with carbonyl-free hexane using nitrogen pressure until the effluent emerged colorless.

Purification of 2,4-Dinitrophenylhydrazones. The effluent from the reaction column contains traces of 2,4-dinitrophenylhydrazine and its decomposition prod-

TABLE I
Conversion of Semicarbazones to 2,4-Dinitrophenylhydrazones

Semicarbazone of:	Concentration, μmoles	Conversion to 2,4-dinitrophenylhydrazone, %
2-Nonadecanone	2.6	102
2-Undecanone	1.7	100
2-Octanone	2.8	101
2-Butanone	2.5	100
Hexadecanal	2.0	94
Tridecanal	1.9	102
Heptanal	2.1	100
Tetradeca-2-enal	2.2	99
2-Ethyl hexa-2-enal	2.3	103
Dodeca-2,4-dienal	2.1	101
Undeca-2,4-dienal	1.9	100
Deca-2,4-dienal	1.5	95

ucts besides the dinitrophenylhydrazone. The latter was isolated from the contaminants by chromatography on alumina. The hexane effluent was passed over a 5 g. column of 6% hydrated alumina as described by Schwartz and Parks. The 2,4-dinitrophenylhydrazone was then selectively eluted with 45 ml. of a 1:1 solution of redistilled benzene: carbonyl-free hexane. The effluent was evaporated under nitrogen on the steam bath. The residue was taken up in chloroform and the concentration of 2,4-dinitrophenylhydrazone determined spectrophotometrically using the maxima and molar extinction coefficients listed by Schwartz, Johnson, and Parks.[13] A blank (hexane-methylene chloride solution) was carried through all steps.

The purity of the 2,4-dinitrophenylhydrazone obtained from the semicarbazone was checked by cochromatographing it with the same concentration of pure, authentic 2,4-dinitrophenylhydrazone derivative in the acetonitrile-hexane system of Corbin, Schwartz, and Keeney.[14]

Results and Discussion

Results of the study are presented in Table I. As the data show, excellent conversion of the semicarbazones to 2,4-dinitrophenylhydrazones was achieved. The chromatographic results revealed that all hydrazones were obtained pure.

The method as described is limited to those semicarbazones which are soluble in methylene chloride–hexane solution. The semicarbazones of acrolein, crotonaldehyde, pentanal, butanal, and propanal were found to be insufficiently soluble to be investigated.

A few experiments under the same conditions were performed on oximes and the 2-diphenylacetyl-1,3,-indanedione 1-hydrazone (azine) derivatives.[15] The oximes

of 2-heptanone, heptanal, and undeca-2-enal were quantitatively converted to 2,4-dinitrophenylhydrazones; the azines of tetradeca-2-enal, acetone, heptanal, and hexadecyl aldehyde were also quantitatively converted.

References

1. Schwartz, D. P., H. S. Haller, and M. Keeney, Abstracts of papers, 136th ACS Meeting, Atlantic City, N. J., 1959, p. 15A.
2. Naves, Y. R., and E. Perrottet, *Helv. Chim. Acta*, **24**, 3 (1941).
3. Naves, Y. R., and A. V. Grampoloff, *Helv. Chim. Acta*, **25**, 1500 (1942).
4. Naves, Y. R., and P. Bachmann, *Helv. Chim. Acta*, **26**, 2151 (1943).
5. Naves, Y. R., *Helv. Chim. Acta*, **29**, 1084 (1946).
6. Naves, Y. R., *Helv. Chim. Acta*, **31**, 2047 (1948).
7. Naves, Y. R., *Helv. Chim. Acta*, **32**, 1798 (1949).
8. Naves, Y. R., and H. Barbier, *Bull. Soc. Chim. France*, Series 5, **20**, 568 (1953).
9. Willhalm, B., U. Steiner, and H. Schinz, *Helv. Chim. Acta*, **41**, 1359 (1958).
10. Nagasawa, K., and S. Ohkuma, *J. Pharm. Soc. Japan*, **74**, 410 (1954); *Chem. Abstr.*, **48**, 8700[b] (1954).
11. Shriner, R. L., and R. C. Fuson, *The Systematic Identification of Organic Compounds*, 3rd ed., Wiley, New York, 1948, p. 170.
12. Schwartz, D. P., and O. W. Parks, *Anal. Chem.*, **33**, 1396 (1961).
13. Schwartz, D. P., A. R. Johnson, and O. W. Parks, *Microchem. J.*, **6** (1962).
14. Corbin, E. A., D. P. Schwartz, and M. Keeney, *J. Chromatog.*, **3**, 322 (1960).
15. Braun, R. A., and W. A. Mosher, *J. Am. Chem. Soc.*, **80**, 3048 (1958).

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